(11)

PATENT SPECIFICATION (21) 34,605/71

Class (52) 87.16

Int. Cl. (51) A61K

Application Number (21) 34605/71
Lodged (22) 14th October, 1971

Complete Specification entitled (54) COMPOSITION AND METHOD FOR TREATMENT OF HEPATIC DISEASE AND MENTAL FATIGUE

Lodged (23) 14th October, 1971
Accepted (44) Lapsed Section 47 c (b)
Published (41) 19th April, 1973

Convention Priority (30) 15th October, 1970, United States of America, 80936

Applicant (71) RICHARDSON-MERRELL S.p.A.

Actual Inventor (72) PROCOLO CAUSA and GIULIO CESARE PERRI

Related Art (56) Nil

The following statement is a full description of this invention, including the best method of performing it known to us :

11746/75-L F. D. Atkinson, Government Printer, Canberra

L203-46-2D-7P.C.

FIELD OF INVENTION

This invention relates to a composition of amino acids and a method for treatment of hepatic diseases and mental fatigue employing the amino acid composition.

DISCUSSION OF PRIOR ART

The association of elevated levels of blood ammonia with hepatic encephalopathies and the possible toxic effects of ammonia in other diseases has been well recognized. Increased amounts of ammonia are introduced into the portal circulation when ammonium salts or a high protein diet are ingested. Extensive hemorrhage into the gastrointestinal tract may produce a similar result. In the presence of impaired hepatic function and/or collateral communications between the portal and systemic veins so common in cirrhosis, the high concentration of ammonia present in the portal blood bypass the hepatic barrier and the ammonia content of the peripheral blood may then be increased to toxic levels. Thus, efforts to reduce blood ammonia are clearly important.

It has been heretofore known that the amino acid L-arginine is beneficial to reduce blood ammonia. The property of arginine to effect a reduction in the ammonia of the blood may be attributed to its role as a precursor of ornithine in the Krebs-Henseleit urea cycle. Arginine is converted in the

liver under the influence of arginase to ornithine and urea. The cycle by which urea is formed involves mostly three amino acids, namely, arginine, ornithine and citrulline. It has likewise been reported that the amino acid L-ornithine is useful to reduce blood ammonia concentration by intravenous infusion when given in amounts three to four times that of the arginine necessary. Also, the amino acid L-citrulline has also been reported to have some beneficial effect although to an even lesser degree.

It has now been found that patients with high blood ammonia levels can be treated with a mixture of L-arginine and L-ornithine in a weight ratio of 3 parts by weight of L-arginine to from 1 to 2 parts by weight of L-ornithine and a synergistic effect is obtained whereby more improved results are obtained from this mixture than from each of L-arginine, L-ornithine or L-citrulline or a mixture of these three amino acids. In fact, with the synergistic mixture of this invention much lower doses of amino acids need be administered and better results are obtained than are obtained with any mixture of the three amino acids or of a single amino acid. The synergistic mixture of amino acids of this invention may be employed in the treatment of hepatic insufficiency, hepatic coma, acute or chronic hepatitis, alone or combined with other therapeutic substances. Moreover, this synergistic mixture of amino acids may be employed in the treatment of mental fatigue, debility and physical and psychic asthenia. The synergistic mixture of amino acids is even more active than acetylglutamine.

SUMMARY OF INVENTION

According to this invention a mixture of 3 parts by weight of L-arginine or a pharmaceutically acceptable acid

addition salt thereof with non-toxic organic or inorganic acids with from 1 to 2 parts by weight of L-ornithine or a pharmaceutically acceptable acid addition salt with a non-toxic organic or inorganic acid is employed in the treatment of hepatic diseases associated with high blood ammonia levels and also for treatment of mental fatigue. A preferred mixture of L-arginine and L-ornithine is one of a mixture of 0.607 mmol L-arginine and 0.169 mmol L-ornithine although other mixtures in the hereinbefore described ratio range are also suitable.

DETAILED DESCRIPTION OF INVENTION

Although the synergistic mixture of amino acids may be employed alone in a sterile, pharmaceutically acceptable carrier, such as water or isotonic salt solution, it is also desirable to combine the synergistic mixture of amino acids with other therapeutic substances, such as, for example, methionine, lipoic acid, cocarboxylase, coenzyme B_{12}, coenzyme A, liver extract and oxybetaine.

As examples of non-toxic, pharmaceutically acceptable acid addition salts of L-arginine and L-ornithine that may be employed in the synergistic mixtures of this invention, there may be mentioned, for example, inorganic acids such as hydrochloric, hydrobromic, sulfuric or phosphoric acids and the like, and organic carboxylic acids such as acetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic, salicylic and the like.

The mixture of this invention can be administered to a host in need of treatment for hepatic diseases and mental fatigue by oral, intravenous or intramuscular administration. Generally, good results are obtained when the mixture is administered to mammals at a daily dosage of from about 7 milli-

grams to about 70 milligrams per kilogram of body weight, preferably given in separate doses two to four times daily. The total daily dosage generally will be from about 500 milligrams to about 12 grams.

The mixture of this invention promotes urea synthesis from ammonia in the liver of a host when administered to said host and thereby lowers the blood level ammonia in the host.

As examples of suitable formulations to be administered to hosts there can be mentioned, for example, the following exemplary formulations.

Oral Administration

(A) Tablets formed by standard tableting techniques from
300 mg L-arginine hydrochloride and
200 mg L-ornithine hydrochloride

(B) Tablets or capsules formed by standard techniques from
125 mg L-arginine phosphate monohydrate,
85 mg L-ornithine phosphate monohydrate
50 mg lipoic acid,
250 mg oxybetaine, and
1 mg coenzyme B_{12}

Intramuscular Administration - solutions of

(A) 300 mg L-arginine hydrochloride and
200 mg L-ornithine hydrochloride
H_2O q.s. to 3 ml

(B) 300 mg L-arginine hydrochloride,
200 mg L-ornithine hydrochloride,
50 mg cocarboxylase,
1 mg coenzyme A,
1 mg coenzyme B_{12}, and
liver extract q.s. to 3 ml

(C) 375 mg L-arginine phosphate monohydrate,
255 mg L-ornithine phosphate monohydrate, and

liver extract q.s. to 3 ml

Phleboclysis and Hypodermoclysis Administration

(A) 3 g L-arginine hydrochloride,

2 g L-ornithine hydrochloride, and

0.6% NaCl solution q.s. to 100 ml

EXAMPLES

The improved results possible by employing the mixture of amino acids instead of single amino acids according to this invention are illustrated in the following examples and Figures 1 to 5 in the drawings as explained hereinafter.

EXAMPLES 1-14

Mice of 18 to 25 grams body weight were injected (i.p.) with 20 ml/kg of the aqueous solution prepared according to the dosages in Tables 1 to 14 and after 30 minutes 20 ml/kg (i.p.) of a 4% NH_4Cl solution (w/v) was injected. Animals were observed for one week; mortality occurred within 3 hours. The results are set forth in the following tables wherein are shown the doses, the number of animals used, the ED_{50} values and their confidence limits.

Table 1

L-ARGININE.HCl PROTECTIVE ACTION AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.	Mortality	%	Log Dose
0.25	18/20	90.0	- 0.6021
0.50	26/40	65.0	- 0.3010
1.00	39/70	55.7	0.0000
2.00	23/50	46.0	0.3010
4.00	4/20	20.0	0.6021

ED_{50} = 1.316 mmol/Kg (0.954 - 1.817) corresponding to 277.23 mg/Kg (200.97 - 382.77) L-Arginine.HCl

Table 2

L-ORNITHINE.HCl PROTECTIVE ACTION AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.	Mortality	%	Log Dose
0.25	19/20	95.0	- 0.6021
0.50	41/60	68.3	- 0.3010
1.00	27/70	38.6	0.0000
2.00	14/50	28.0	0.3010
4.00	1/20	5.0	0.6021

ED_{50} = 0.857 mmol/Kg (0.713 - 1.032) corresponding to 144.54 mg/Kg (120.23 - 174.02) L-Ornithine.HCl

Table 3

L-CITRULLINE PROTECTIVE ACTION AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.	Mortality	%	Log Dose
0.25	30/40	75.0	- 0.6021
0.50	41/60	68.3	- 0.3010
1.00	48/90	53.3	0.0000
2.00	27/70	38.6	0.3010
4.00 *	1/40	2.5	

* The data relative to this dose have not been used in the probit calculations.

ED_{50} = 1.026 mmol/Kg (0.817 - 1.949) corresponding to 179.74 mg/Kg (143.13 - 341.45) L-Citrulline

Table 4

PROTECTIVE ACTION OF A L-ARGININE.HCl (81.79% W/W) AND L-ORNITHINE.HCl (18.21% W/W) MIXTURE - MIXTURE A - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCl	0.4746	23/40	57.5	- 0.3237
L-Ornithine.HCl	0.1304			
L-Arginine.HCl	0.5316	22/40	55.0	- 0.2744
L-Ornithine.HCl	0.1482			
L-Arginine.HCl	0.5981	29/40	72.5	- 0.2232
L-Ornithine.HCl	0.1660			
L-Arginine.HCl	0.6693	19/40	47.5	- 0.1743
L-Ornithine.HCl	0.1838			
L-Arginine.HCl	0.7500	13/40	32.5	- 0.1249
L-Ornithine.HCl	0.2075			
L-Arginine.HCl	0.8449	9/40	22.5	- 0.0732
L-Ornithine.HCl	0.2372			
L-Arginine.HCl	0.9493	7/40	17.5	- 0.0226
L-Ornithine.HCl	0.2668			
L-Arginine.HCl	1.0680	5/40	12.5	0.0286
L-Ornithine.HCl	0.2965			

ED_{50} = 0.607 mmol/Kg L-Arginine.HCl (0.561 - 0.656) associated with 0.169 mmol/Kg L-Ornithine.HCl (0.156 - 0.182) corres. to 127.87 mg/Kg L-Arginine.HCl (118.18 - 138.19) assoc. with 28.50 mg/Kg L-Ornithine.HCl (26.30 - 30.69)

Table 5

PROTECTIVE ACTION OF A L-ARGININE.HCl (59.90% W/W) AND L-ORNITHINE.HCl (40.10% W/W) MIXTURE - MIXTURE B - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCl	0.1898	15/20	75.0	- 0.7216
L-Ornithine.HCl	0.1601			
L-Arginine.HCl	0.2990	28/40	70.0	- 0.5243
L-Ornithine.HCl	0.2490			
L-Arginine.HCl	0.3797	22/40	55.0	- 0.4206
L-Ornithine.HCl	0.3202			
L-Arginine.HCl	0.4746	17/40	42.5	- 0.3237
L-Ornithine.HCl	0.3973			
L-Arginine.HCl	0.5933	3.20	15.0	- 0.2267
L-Ornithine.HCl	0.4981			
L-Arginine.HCl	0.7500	2/20	10.0	- 0.1249
L-Ornithine.HCl	0.6286			

ED_{50} = 0.383 mmol/Kg L-Arginine.HCl (0.340 - 0.432) assoc. with 0.320 mmol/Kg L-Ornithine.HCl (0.284 - 0.361) corres. to 80.68 mg/Kg L-Arginine.HCl (71.62 - 91.01) assoc. with 53.96 mg/Kg L-Ornithine.HCl (47.89 - 60.87)

34605 m

Table 6

PROTECTIVE ACTION OF A L-ARGININE.HCl (33.63% W/W) AND L-ORNITHINE.HCl (66.37% W/W) MIXTURE - MIXTURE C - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCl	0.2990	30/40	75.0	- 0.5243
L-Ornithine.HCl	0.7413			
L-Arginine.HCl	0.3370	13/20	65.0	- 0.4724
L-Ornithine.HCl	0.8302			
L-Arginine.HCl	0.3750	15/20	75.0	- 0.4260
L-Ornithine.HCl	0.9310			
L-Arginine.HCl	0.4224	15/20	75.0	- 0.3743
L-Ornithine.HCl	1.0437			
L-Arginine.HCl	0.4746	25/40	62.5	- 0.3237
L-Ornithine.HCl	1.1683			
L-Arginine.HCl	0.5316	21/40	52.5	- 0.2744
L-Ornithine.HCl	1.3106			
L-Arginine.HCl	0.5981	11/40	27.5	- 0.2232
L-Ornithine.HCl	1.4707			
L-Arginine.HCl	0.6693	8/40	20.0	- 0.1743
L-Ornithine.HCl	1.6486			
L-Arginine.HCl	0.7500 *	3/40	7.5	
L-Ornithine.HCl	1.8562			

* The data relative to this dose have not been used in the probit calculations.

ED_{50} = 0.490 mmol/Kg L-Arginine.HCl (0.453 - 0.529) assoc. with 1.208 mmol/Kg L-Ornithine.HCl (1.117 - 1.304) corres. to 103.22 mg/kg L-Arginine.HCl (95.43- 111.44) assoc. with 203.69 mg/Kg L-Ornithine.HCl (188.35 - 219.88)

34.605/71

Table 7

PROTECTIVE ACTION OF A L-ARGININE.HCl (75% W/W) AND A L-CITRULLINE (25% W/W) MIXTURE - MIXTURE A' - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCL	0.2990	33/40	82.5	- 0.5243
L-Citrulline	0.1198			
L-Arginine.HCl	0.4746	30/40	75.0	- 0.3237
L-Citrulline	0.1883			
L-Arginine.HCl	0.7500	21/40	52.5	- 0.1249
L-Citrulline	0.3025			
L-Arginine.HCl	1.1914	15/40	37.5	0.0760
L-Citrulline	0.4794			

ED_{50} = 0.848 mmol/Kg L-Arginine.HCl (0.662 - 1.085) assoc.
with 0.340 mmol/Kg L-Citrulline (0.265 - 0.435) corres.
to 178.65 mg/Kg L-Arginine.HCl (139.46 - 228.57) assoc.
with 59.55 mg/Kg L-Citrulline (46.49 - 76.19)

Table 8

PROTECTIVE ACTION OF A L-ARGININE.HCl (50% W/W) AND A L-CITRULLINE (50% W/W) MIXTURE - MIXTURE B' - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCl	0.2373	31/40	77.5	- 0.6247
L-Citrulline	0.2854			
L-Arginine.HCl	0.3750	23/40	57.5	- 0.4260
L-Citrulline	0.4509			
L-Arginine.HCl	0.5981	21/40	52.5	- 0.2232
L-Citrulline	0.7192			
L-Arginine.HCl	0.9493	16/40	40.0	- 0.0226
L-Citrulline	1.1416			

ED_{50} = 0.625 mmol/Kg L-Arginine.HCl (0.450 - 0.867) assoc.
with 0.751 mmol/Kg L-Citrulline (0.541 - 1.042) corres.
to 131.66 mg/Kg L-Arginine.HCl (94.80 - 182.64) assoc.
with 131.66 mg/Kg L-Citrulline (94.80 - 182.64)

Table 9

PROTECTIVE ACTION OF A L-ARGININE.HCl (25% W/W) AND A L-CITRULLINE (75% W/W) MIXTURE - MIXTURE C' - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Arginine Log Dose
L-Arginine.HCl	0.1898	29/40	72.5	- 0.7216
L-Citrulline	0.6792			
L-Arginine.HCl	0.2373	20/40	50.0	- 0.6247
L-Citrulline	0.8562			
L-Arginine.HCl	0.2990	16/40	40.0	- 0.5243
L-Citrulline	1.0788			
L-Arginine.HCl	0.3750	13/40	32.5	- 0.4260
L-Citrulline	1.3585			

ED_{50} = 0.249 mmol/Kg L-Arginine.HCl (0.227 - 0.272) assoc.
with 0.898 mmol/Kg L-Citrulline (0.819 - 0.981) corres.
to 52.45 mg/Kg L-Arginine.HCl (47.82 - 57.30) assoc.
with 157.35 mg/Kg L-Citrulline (143.46 - 171.91)

Table 10

PROTECTIVE ACTION OF A L-CITRULLINE (75% W/W) AND A L-ORNITHINE.HCl (25% W/W) MIXTURE - MIXTURE A" - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Citrulline Log Dose
L-Citrulline	0.4566	33/40	82.5	- 0.3404
L-Ornithine.HCl	0.1601			
L-Citrulline	0.5708	25/40	62.5	- 0.2435
L-Ornithine.HCl	0.1957			
L-Citrulline	0.7192	25/40	62.5	- 0.1432
L-Ornithine.HCl	0.2490			
L-Citrulline	0.9018	31/60	51.7	- 0.0449
L-Ornithine.HCl	0.3143			
L-Citrulline	1.1416	10/40	25.0	0.0576
L-Ornithine.HCl	0.3973			
L-Citrulline	1.4327	6/40	15.0	0.1561
L-Ornithine.HCl	0.4922			

ED_{50} = 0.815 mmol/Kg L-Citrulline (0.745 - 0.893) assoc.
with 0.282 mmol/Kg L-Ornithine.HCl (0.258 - 0.309) corres.
to 142.78 mg/Kg L-Citrulline (130.52 - 156.44) assoc.
with 47.59 mg/Kg L-Ornithine.HCl (43.51 - 52.15)

Table 11

PROTECTIVE ACTION OF A L-CITRULLINE (50% W/W) AND A L-ORNITHINE.HCl (50% W/W) MIXTURE - MIXTURE B" - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Citrulline Log Dose
L-Citrulline	0.2854	28/40	70.0	- 0.5446
L-Ornithine.HCl	0.2965			
L-Citrulline	0.4566	26/40	65.0	- 0.3404
L-Ornithine.HCl	0.4744			
L-Citrulline	0.5080	27/40	67.5	- 0.2941
L-Ornithine.HCl	0.5278			
L-Citrulline	0.5708	23/40	57.5	- 0.2435
L-Ornithine.HCl	0.5930			
L-Citrulline	0.6393	16/40	40.0	- 0.1943
L-Ornithine.HCl	0.6642			
L-Citrulline	0.7192	8/40	20.0	- 0.1432
L-Ornithine.HCl	0.7472			
L-Citrulline	1.1416	5/40	12.5	0.0566
L-Ornithine.HCl	1.1860			

ED_{50} = 0.539 mmol/Kg L-Citrulline (0.483 - 0.602) assoc.
with 0.560 mmol/Kg L-Ornithine.HCl (0.502 - 0.625) corres.
to 94.43 mg/Kg L-Citrulline (84.62 - 105.46) assoc.
with 94.43 mg/Kg L-Ornithine.HCl (84.62 - 105.46)

Table 12

PROTECTIVE ACTION OF A L-CITRULLINE (25% W/W) AND L-ORNITHINE.HCl (75% W/W) MIXTURE - MIXTURE C" - AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mmol/Kg i.p.		Mortality	%	Citrulline Log Dose
L-Citrulline	0.1141	36/40	90.00	- 0.9427
L-Ornithine.HCl	0.3558			
L-Citrulline	0.1826	32/40	80.00	- 0.7384
L-Ornithine.HCl	0.5693			
L-Citrulline	0.2283	19/40	47.50	- 0.6415
L-Ornithine.HCl	0.7116			
L-Citrulline	0.2854	32/60	53.33	- 0.5446
L-Ornithine.HCl	0.8895			
L-Citrulline	0.3596	39/60	65.00	- 0.4454
L-Ornithine.HCl	1.1208			
L-Citrulline	0.4509	31/80	38.75	- 0.3459
L-Ornithine.HCl	1.4114			
L-Citrulline	0.5708	5/40	12.50	- 0.2435
L-Ornithine.HCl	1.7791			

ED_{50} = 0.330 mmol/Kg L-Citrulline (0.296 - 0.369) assoc.
with 1.028 mmol/Kg L-Ornithine.HCl (0.922 - 1.149) corres.
to 57.81 mg/Kg L-Citrulline (51.86 - 64.65) assoc.
with 173.43 mg/Kg L-Ornithine.HCl (155.58 - 193.95)

Table 13

PROTECTIVE ACTION OF L-ARGININE.HCl AND L-ORNITHINE.HCl MIXTURE IN THE RATIO 3:2 ASSOCIATED WITH L-CITRULLINE AT CRESCENT DOSES AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

		Composition mg/Kg	Composition %	Mortality	ED found	Theoretic ED
(a)	L-Arginine.HCl	100.00	52.10	25/40	37.5	50
	L-Ornithine.HCl	66.95	34.88			
	L-Citrulline	25.00	13.02			
		191.95				
(b)	L-Arginine.HCl	100.00	46.09	30/40	25	50
	L-Ornithine.HCl	66.95	30.86			
	L-Citrulline	50.00	23.05			
		216.95				
(c)	L-Arginine.HCl	100.00	37.46	31/40	22.5	50
	L-Ornithine.HCl	66.95	25.08			
	L-Citrulline	100.00	37.46			
		266.95				
(d)	L-Arginine.HCl	100.00	27.25	22/40	45	50
	L-Ornithine.HCl	66.95	18.25			
	L-Citrulline	200.00	54.50			
		366.95				
(e)	L-Arginine.HCl	100.00	17.64	31/40	22.5	50
	L-Ornithine.HCl	66.95	11.81			
	L-Citrulline	400.00	70.55			
		566.95				
(f)	L-Arginine.HCl	100.00	10.34	29.40	27.5	50
	L-Ornithine.HCl	66.95	6.92			
	L-Citrulline	800.00	82.74			
		966.95				

Table 14

PROTECTIVE ACTION OF A L-ARGININE.HCl (50%), A L-ORNITHINE.HCl (25%), AND A L-CITRULLINE (25%) MIXTURE AGAINST ACUTE MOUSE INTOXICATION BY NH_4Cl

Dose mg/Kg		Mortality	%	Arginine Log Dose
L-Arginine.HCl	79.4	36/50	72	1.90
L-Ornithine.HCl	39.7			
L-Citrulline	39.7			
L-Arginine.HCl	100.0	30/50	60	2.00
L-Ornithine.HCl	50.0			
L-Citrulline	50.0			
L-Arginine.HCl	112.2	31/50	62	2.05
L-Ornithine.HCl	56.1			
L-Citrulline	56.1			
L-Arginine.HCl	125.9	18/50	36	2.10
L-Ornithine.HCl	63.0			
L-Citrulline	63.0			
L-Arginine.HCl	141.3	12/50	24	2.15
L-Ornithine.HCl	70.6			
L-Citrulline	70.6			

Table 14 (Continued)

ED_{50} =	110.80 mg/Kg	L-Arginine.HCl	(104.00 - 118.10) to which are associated
	55.40 mg/Kg	L-Ornithine.HCl	(52.00 - 59.05)
	55.40 mg/Kg	L-Citrulline	(52.00 - 59.05)
Total	221.60 mg/Kg		(208.00 - 236.20)

In order to more fully show the activity of each component in the mixtures of the tables, the results are illustrated in Figures 1 to 5 of the drawings by the graphic representation proposed by Loewe, Arzneim. Forsch 3 285 (1953); Loewe, Pharmacol. Rev. 9 237 (1957) and Loewe, Arzneim. Forsch 9 449 (1959). The mg/Kg doses of one of the components are indicated on the ordinates and of the other component, on the abscissae. On the coordinates are indicated the doses of each single component determining any effect quantitatively or qualitatively similar, that is, ED_{50}.

Referring to Figure 1, the resulting lines have been called "isoboles" by Loewe. If the effect is due to activities common to both components, that is, toxicity, in this case the lines can:

1. not be affected reciprocally (line a and b);
2. sum up (line c);
3. potentiate (curve d);
4. antagonize (curve e and f).

The antagonism can be absolute when the isobole goes beyond the right angle formed by the two doses with equal action of the two non-mixed components (curve f) or relative when the isobole is convex but keeping within the right angle (curve e).

Figures 2, 3 and 4 show the isoboles protecting mortality induced in mice by NH_4Cl (ED_{50}) of mixtures of L-arginine.HCl + L-ornithine.HCl (Figure 2); L-arginine.HCl + L-citrulline (Figure 3); and L-citrulline + L-ornithine.HCl (Figure 4).

Figure 2 shows that in L-arginine.HCl + L-ornithine.HCl

mixtures there is at first a synergism with strengthening till a determine dosage relation; then an absolute antagonism.

Figure 3 shows that in L-arginine.HCl + L-citrulline mixtures there is an effect similar to the addition of effects; then a relative antagonism.

Figure 4 shows that in L-citrulline + L-ornithine.HCl mixtures there is only antagonism, at first relative, and then absolute.

These results indicate that in the study of the two component mixtures, the L-arginine.HCl + L-ornithine.HCl mixture gives the best results when mixed in a 3:2 ratio as shown by 80.68 mg/Kg L-arginine.HCl + 53.96 mg/Kg L-ornithine.HCl; see Table 5 and Figure 2.

We did not modify this ratio and in further experiments, L-arginine.HCl + L-ornithine.HCl 3:2 mixture was considered as a single substance, to which L-citrulline was added. The results obtained show that the addition of L-citrulline decreases the protective action; see Table 13 and Figure 5. The ordinate shows the L-arginine.HCl + L-ornithine.HCl mixture in 3:2 ratio, while the abscissa shows L-citrulline. The radii, starting from the origin of coordinates, correspond to the different mixtures, the composition of which is shown in Table 13.

The circle arc connecting the ordinate (100% L-arginine.HCl + L-ornithine.HCl 3:2 mixture) to the abscissa (100% L-citrulline) is the line of the ED_{50} theoretically calculated according to the sum of the effects, while the above curve represents the ED we found. Each radius, intersecting the curve of the ED_{50}, is divided in proportional parts: ED_{100} starts at the origin of coordinates, while ED_0 is set at a distance from the intersection point, equal to that between the origin of the coordinates and the intersection point.

The ED_{50} of our L-arginine.HCl + L-ornithine.HCl 3:2 mixture corresponds to 134.64 mg/Kg of the total aminoacid which is 39.24% less than the sum of each aminoacid.

EXAMPLE 15

To illustrate the protective action of the mixture of this invention in the treatment of mental fatigue, mice of 18 to 25 grams body weight were injected with 20 ml/kg of aqueous solution according to the dosages in Table 15 and after 30 minutes a single intraventicular injection of 0.03 ml 4% NH_4Cl (w/v) was injected into each animal. Animals were observed for one week; mortality occurred within 3 hours.

Table 15 shows that pretreatment with L-arginine.HCl or L-ornithine.HCl has a poor protective action, although statistically significant, based on mortality induced by NH_4Cl injected intercerebrally (Chi square test with Yates correction). L-arginine.HCl + L-ornithine.HCl 3:2 mixture has a clearly higher protective action degree at lower doses than with each aminoacid.

Table 15

Protective action of L-arginine.HCl, L-ornithine.HCl and of a mixture in 3:2 ratio in acute intoxication induced in mice by NH_4Cl intracerebral injection.

Substance	Dose (mg/kg)	Mortality	Protective Action %	Chi Square	P
Physiol. sol.	10 ml/kg i.p.	50/50	0.00	--	--
L-Arginine.HCl	400 mg/kg i.p.	32/40	20.00	8.645	0.001 0.005
L-Arginine.HCl	800 mg/kg i.p.	31/40	22.50	10.125	0.001 0.005
L-Ornithine.HCl	400 mg/kg i.p.	31/40	22.50	10.125	0.001 0.005
L-Ornithine.HCl	800 mg/kg i.p.	33/40	17.50	7.205	0.005 0.010
L-Arginine.HCl + L-Ornithine.HCl	400 mg/kg i.p. + 267 mg/kg i.p.	23/40	42.50	23.498	0.005

EXAMPLE 16

We have tested L-arginine.HCl, L-ornithine.HCl and L-arginine.HCl + L-ornithine.HCl mixture in 3:2 ratio in the protective action against acute intoxication induced in mice by NH_4Cl intracerebrally injected (0.03 ml/mouse of 4% NH_4Cl), in comparison with L-acetylglutamine. These substances were administered to mice of both sexes, 18-25 grams body weight (i.p.) 1 hour before the intraventricular injection of 4% NH_4Cl as summarized in Table 16.

Table 16

Protective Substance	Dose (mg/kg)	Mortality induced by NH_4Cl intracerebrally injected, after 1 hour: Total Deaths Number	% Mortality
L-Acetylglutamine	100	38/40	95.0
	200	34/40	85.0
	400	33/40	82.5
	800	31/40	77.5
	1600	28/40	70.0
L-Arginine.HCl	100	36/40	90.0
	200	34/40	85.0
	400	32/40	80.0
	800	31/40	77.5
	1600	27/40	67.5
L-Ornithine.HCl	100	36/40	90.0
	200	34/40	85.0
	400	31/40	77.5
	800	33/40	82.5
	1600	29/40	72.5
L-Arginine.HCl + L-Ornithine.HCl in 3:2 ratio	167	31/40	77.5
	334	28/40	70.0
	668	23/40	57.5
	1356	25/40	62.5

(Doses are referred to the sum of the two aminoacids).

Having set L-acetylglutamine potency equal to 1:00 (standard) the other substances are set in the following order:

Table 17

Substance		Potency	Confidence Limits (P = 0.05)
L-Acetylglutamine		1.000	
L-Arginine.HCl		1.337	(P.441 - 4.046)
L-Ornithine.HCl		1.094	(0.350 - 3.322)
L-Arginine.HCl + L-Ornithine.HCl	3:2	5.676	(1.195 - 26.970)

The above-mentioned results are shown in Figure 6. L-arginine.HCl and L-ornithine.HCl have a good protective action against ammonia intoxication intracerebrally performed in mice. Their effectiveness, however, is significantly similar to that shown by a standard substance (L-acetylglutamine).

The L-arginine.HCl + L-ornithine.HCl 3:2 mixture, instead is considerably more active than the reference substance and this activity ratio is highly significant, since the inferior confidence limit is higher than unity.

The claims defining the invention are as follows:

1. A composition for treatment of hepatic diseases and mental fatigue comprising a pharmaceutically acceptable carrier and a mixture of 3 parts by weight of L-arginine or a pharmaceutically acceptable acid salt thereof and 2 parts by weight of L-ornithine or a pharmaceutically acceptable acid salt thereof.

2. The composition of Claim 1 comprising a mixture of 0.607 mol L-arginine or salt thereof and 0.169 mol L-ornithine or salt thereof.

3. The composition of Claim 1 containing one or more additional therapeutic substances.

4. The composition of Claim 3 wherein the additional therapeutic substances are selected from methionine, lipoic acid, cocarboxylase, coenzyme B_{12}, coenzyme A, liver extract and oxybetaine.

5. The composition of Claim 4 which is dissolved in liver extract.

6. The composition of Claim 1 wherein the mixture comprises 3 parts of L-arginine hydrochloride and 2 parts L-ornithine hydrochloride.

7. The composition of Claim 2 wherein the L-arginine and the L-ornithine are present as their hydrochloride salts.

8. A method for the treatment of hepatic diseases and mental fatigue comprising administering to a host in need of such treatment a composition comprising a pharmaceutically acceptable carrier and a mixture of 3 parts by weight of

L-arginine or a pharmaceutically acceptable acid salt thereof and 2 parts by weight of L-ornithine or a pharmaceutically acceptable acid salt thereof.

9. The method of Claim 8 wherein the composition is administered by an intravenous administration.

10. The method of Claim 8 wherein the composition is administered by an oral administration.

11. The method of Claim 8 wherein the composition is administered by an intramuscular administration.

12. The method of Claim 8 wherein from 7 to 70 milligrams per kilogram of host body weight of the mixture is administered to the host.

13. A method of promoting urea synthesis from ammonia in the liver of a host comprising administering to said host a composition comprising a pharmaceutically acceptable carrier and a mixture of 3 parts by weight L-arginine or a pharmaceutically acceptable acid salt thereof and 2 parts by weight of L-ornithine or a pharmaceutically acceptable acid salt thereof.

DATED: 4th October, 1971

PHILLIPS ORMONDE AND FITZPATRICK
Attorneys for:
RICHARDSON-MERRELL S.p.A.

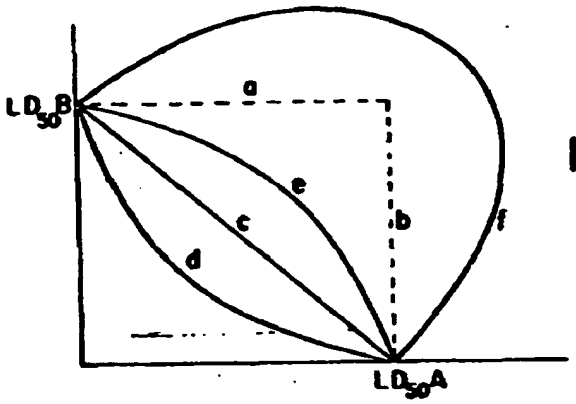

FIG. 1

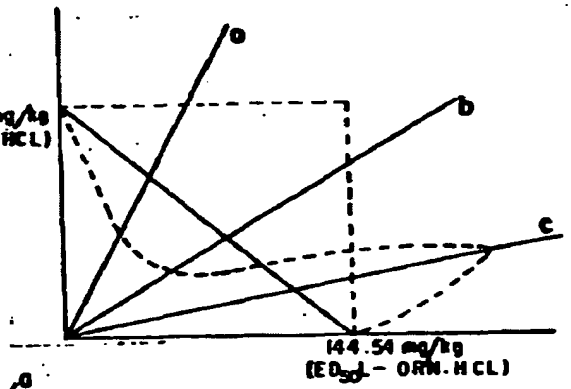

FIG. 2

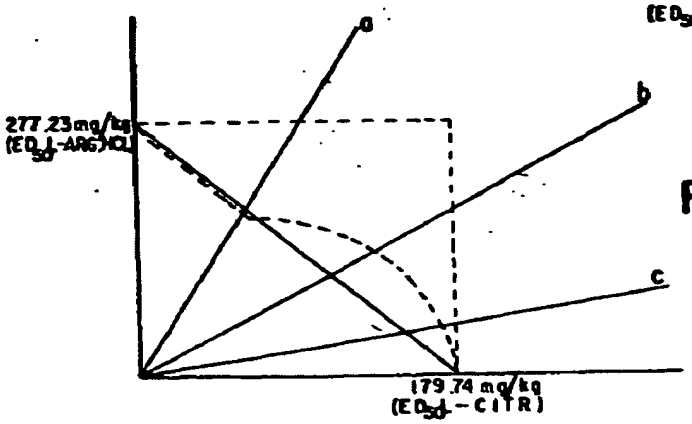

FIG. 3

34.605m

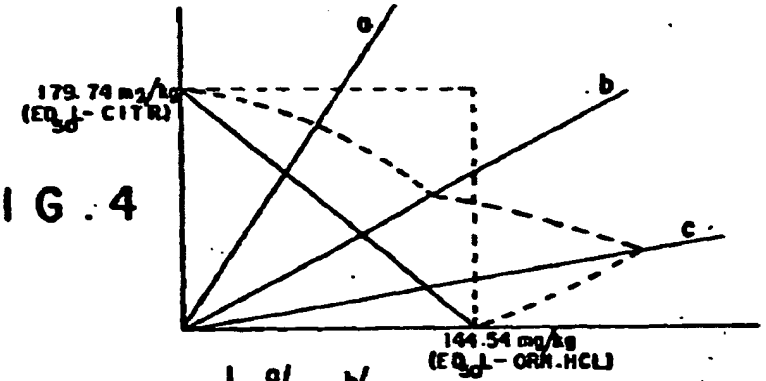

FIG. 4

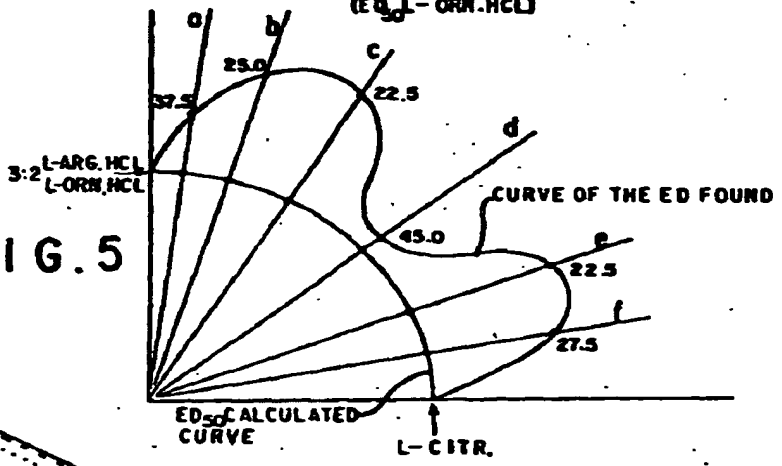

FIG. 5

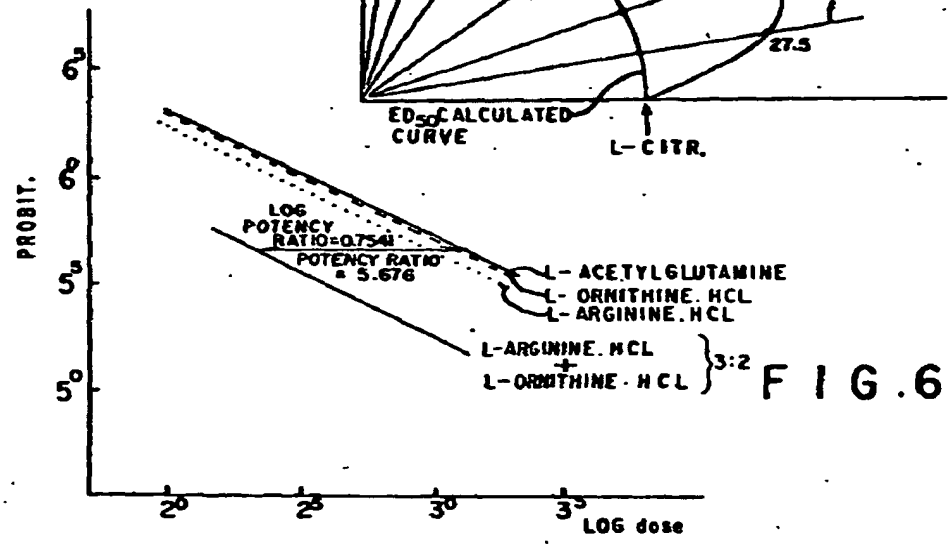

FIG. 6

34.605 m

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.